Membrane Attack Complex Deposition in Experimental Glomerular Injury

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The complement (C) system is an important mediator of glomerular injury both through its attraction of inflammatory cells and by a cell-independent effect on glomerular capillary wall permeability. We have postulated that the latter effect may be mediated by the terminal components of the C system, the membrane attack complex (MAC). We examined several models of immunologic renal injury in the rat by immunofluorescence for the presence of neoantigens of the MAC. Rats with experimental membranous nephropathy induced by antibody binding to a fixed glomerular antigen (passive Heymann nephritis, PHN) or a planted antigen (autologous phase of PHN) had moderate proteinuria and 1–2+ capillary wall deposits of IgG, rat C3, and MAC. C depletion with cobra venom factor (CVF) significantly decreased proteinuria and prevented deposition of C3 and MAC. Rats with active Heymann nephritis had similar capillary wall deposits of MAC. Rats with anti-glomerular basement membrane nephritis developed severe proteinuria which was not affected by CVF treatment and had no glomerular deposits of MAC. Rats with nonimmunologic proteinuria induced by aminonucleoside of puromycin also had no glomerular deposits of MAC. In rats unilaterally nephrectomized before the induction of PHN segmental glomerular sclerosis developed after 6 months with deposits of MAC in the sclerotic areas. The presence or absence of glomerular deposits of MAC in experimental renal disease correlates well with the pathogenetic role of C in the production of injury. These results support a role for the MAC in the mediation of several types of glomerular injury. (Am J Pathol 1985, 120:121–128)

THE COMPLEMENT SYSTEM has long been recognized as an important mediator of immunologically induced glomerular injury.[1-3] Early studies of its mechanism of action were done predominantly in models of nephrotoxic or anti-glomerular basement membrane (GBM) nephritis and suggested an indirect role for complement.[3-6] This involved the attraction of polymorphonuclear leukocytes by chemotactic peptides, mainly C5a, and possibly immune adherence via C3b receptors.[2,3] Capillary wall damage was postulated to be due to the release of neutrophil enzymes which have been shown to be capable of digesting GBM *in vitro*.[7]

We have recently shown that the complement system also plays a direct role in producing glomerular capillary wall injury that does not involve the participation of cellular mediators.[8,9] Proteinuria in experimental membranous nephropathy in the rat induced by antibody reacting with either fixed or planted glomerular antigens is highly complement-dependent but is unaffected by depletion of inflammatory cells. We speculated that this might involve the lytic action of the terminal complement components acting on some element of the filtration barrier.[8,9] Subsequent studies in C6-deficient rabbits demonstrated a marked reduction in proteinuria in a model of membranous nephropathy induced by repeated injections of cationic bovine serum albumin (cBSA) and in the heterologous phase of anti-GBM nephritis.[10,11] These studies provided the first evidence of the functional participation of terminal complement components in the production of capillary wall injury.

Further support for this hypothesis comes from

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studies of a variety of human renal diseases and of serum sickness in the rat, in which glomerular deposition of neoantigens of the membrane attack complex (MAC) has been demonstrated.[12-15] However, the role of complement in producing the lesions studied is unknown, and the presence of MAC neoantigens in presumably non-immune-mediated lesions raises the possibility of secondary deposition of complement components in injured tissue. Using antibodies to the terminal complement components C5, C6, C7, and C8, we have recently shown that the presence or absence of these components in glomerular immune deposits in several models of renal injury in the rat correlates well with the complement-dependent nature of the lesion.[16] We now report on the production of antibodies to the neoantigens of the rat MAC and confirm the relationship between deposition of MAC neoantigens and the role for complement in producing the lesion in several models of immune and nonimmune glomerular injury. These findings provide further support for the hypothesis that the MAC is an important mediator of immune glomerular injury.

Materials and Methods

Preparation of Rat MAC

Rat MAC was prepared following a modification of the method of Biesecker et al for the preparation of human MAC.[17] Human C5 isolated to homogeneity, as assessed by SDS-polyacrylamide gel electrophoresis,[18] was iodinated with ^{125}I with the use of Iodo-beads (Pierce Chemical Co., Rockford, Ill). Rabbit erythrocytes (RBCs) were isolated from whole rabbit blood collected into 10 mM EDTA, washed four times with veronal buffered saline (VBS) containing 5 mM EDTA and three times with VBS containing 1% (wt/vol) gelatin plus 0.5 mM $MgCl_2$ and 0.15 mM $CaCl_2$.

Packed rabbit RBCs (5.5×10^{10}) were suspended in 110 ml of fresh rat serum containing 0.1 mg of ^{125}I-human C5 and 5 mM Mg^{++} and incubated at 37 C in a shaking water bath for 30 minutes. Cell lysis occurs as a result of activation of the alternative complement pathway by the rabbit RBC membrane.[19] The RBC membranes were harvested by centrifugation (10,000 *g*, 30 minutes), washed twice in 5 mM EDTA, pH 8.0, and resuspended in 3 ml of 10% deoxycholate (DOC), 50 mM Tris-acetate, pH 8.9. They were extracted for 2 hours with continuous stirring at room temperature. The extract was centrifuged for 1 hour at 100,000 *g* (Beckman Model L, Beckman Instruments, Inc., Fullerton, Calif). The MAC was isolated by gel filtration on Sepharose CL-6B (Pharmacia Fine Chemicals, Piscataway, NJ) in 2% DOC, 50 mM Tris-acetate, pH 8.9. Fractions were counted for ^{125}I in a gamma counter (Packard Instrument Co., Downers Grove, Ill). Complex containing fractions were pooled on the basis of radioactivity, concentrated to approximately 1 mg/ml in a collodion bag apparatus (Schleicher and Schuell, Inc., Keene, NH), and dialyzed against phosphate-buffered saline (PBS), pH 7.2.

Production and Purification of Polyclonal Rabbit Anti-Rat MAC

Male New Zealand white rabbits weighing 2 kg (R and R Rabbitry, Stanwood, Wash) were immunized with 0.5 mg of rat MAC at a concentration of 0.5 mg/ml emulsified with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich) and given into the popliteal fossae and intradermally in multiple sites on the back. Animals were boosted with the same dose emulsified in incomplete Freund's adjuvant at 3-week intervals and bled 1 week after each boost. All animals were exsanguinated after 3 months.

The globulin fraction of antiserum was precipitated with an equal volume of saturated ammonium sulfate, washed twice with 50% ammonium sulfate, redissolved in one-half the original volume of PBS, and dialyzed against PBS. Normal rat plasma containing 40 mM EDTA was coupled to CNBr-activated Sepharose-4B (Pharmacia Fine Chemicals) as outlined by the manufacturer. Rabbit anti-rat MAC globulin (40–50 mg) was passed over a column containing 50 ml of coupled gel. The protein passing through the column was concentrated with an Amicon PM-30 membrane (Amicon Corp., Scientific Systems Division, Danvers, Mass) to a final connection of approximately 30 mg/ml.

Rabbit anti-rat MAC was tested by micro-Ouchterlony immunodiffusion and immunoelectrophoresis in 1% agarose gel containing 10 mM EDTA against rat MAC (1 mg/ml), normal rat plasma containing 40 mM EDTA, and zymosan-activated rat serum. Specificity for rat MAC was further assessed by absorption studies as outlined below. Rabbit anti-rat MAC antibody was used in indirect immunofluorescence of rat kidney tissue as outlined below.

Models of Glomerular Disease

Passive Heymann Nephritis (PHN—Heterologous Phase)

Eight male Sprague-Dawley rats (Tyler Laboratories, Inc., Bellevue, Wash) were given injections of 1.25 ml of sheep anti-rat Fx1A antiserum intravenously[9,16,20] prepared by hyperimmunization of sheep with rat proximal tubular epithelial cell brush border antigen (Fx1A).[21] Three of the rats were given purified cobra

venom factor (CVF, Cordis Laboratories, Miami, Fla), 300 U/kg intraperitoneally followed by 100 U/kg/d intraperitoneally for 5 days for depletion of circulating complement components.[1] Urine was collected for determination of protein excretion from Day 4–5 following antiserum injection, and tissue for immunofluorescence studies was obtained on Day 5. Staining of this tissue for terminal complement components (C5, C6, C7, C8) has been reported elsewhere.[16]

γ_2 PHN (Autologous Phase)

Non-complement-fixing (γ_2 subclass) sheep anti-rat Fx1A IgG was isolated as previously described.[8,9] Injection of γ_2 anti-Fx1A IgG alone does not produce proteinuria but results in subepithelial deposits of γ_2 sheep IgG, which act as a planted, exogenous antigen. To study the complement-dependent proteinuria which develops when autologous antibody binds to these deposits, we gave 7 rats injections of 8.1 mg of γ_2-anti-Fx1A IgG intravenously 5 days after immunization with 1.0 mg of sheep IgG in complete Freund's adjuvant (Difco Laboratories).[8,16] Proteinuria develops 7 days after injection of γ_2-anti-Fx1A and is associated with subepithelial deposits of sheep IgG, rat anti-sheep IgG antibody, and rat C3.[8] Three animals were treated with CVF, as outlined above, for 8 days for depletion of circulating complement components. Urine was collected for determination of protein excretion and tissue for immunofluorescence studies was obtained on Day 7–8 following antibody injection. Staining of this tissue for terminal complement components (C5, C6, C7, C8) has been reported elsewhere.[16]

Autologous Immune Complex Nephropathy (AICN)

Three male Lewis rats (150–200 g; Simonsen Laboratories, Inc., Gilroy, Calif) were immunized with 5 mg of Fx1A emulsified in complete Freund's adjuvant as described previously.[22] Urine was collected for determination of protein excretion and immunofluorescence studies were carried out 7 weeks after immunization. Staining of this tissue for terminal complement components (C5, C6, C7, C8) has been reported elsewhere.[16]

Nephrotoxic Nephritis (NTN-Heterologous Phase)

Three rats were given injections of 0.125 ml of sheep anti-rat GBM antiserum IV, prepared as described previously.[16,23,24] Urine was collected for determination of protein excretion during the 24-hour period after antiserum injection, and tissue for immunofluorescence study was obtained 24 hours after antiserum injection.

Aminonucleoside Nephrosis

A nonimmune model of glomerular disease with proteinuria was produced in 3 rats by injection of aminonucleoside of puromyucin (PA; ICM Pharmacaeuticals, Inc., Life Sciences Group, Cleveland, OH) 100 mg/kg intravenously, as described previously.[16,25] Urine was collected for determination of protein excretion, and renal tissue was obtained for immunofluorescence studies 9 days after PA injection. Staining of this tissue for terminal complement components (C5, C6, C7, C8) has been reported elsewhere.[16]

Segmental Sclerosis

To obtain a model of sclerotic lesions in the rat, we unilaterally nephrectomized the animals before the induction of PHN. Six rats weighing 200–225 g had a single kidney removed under ether anesthesia, after ligation of the renal pedicle, and 24 hours later were given injections of 2.0 ml of sheep anti-rat Fx1A. Urine protein excretion and tissue for immunofluorescence studies was obtained after 24 weeks.

Immunofluorescence Microscopy

Tissue for immunofluorescence was obtained by open renal biopsy under ether anesthesia or at sacrifice and snap-frozen in dry-ice–isopentane. Cryostat sections were cut at 4 μ, air-dried, and fixed in ether-alcohol as described elsewhere.[25,26]

Rabbit antibody to rat MAC was titered by indirect immunofluorescence on PHN tissue. Serial twofold dilutions of antibody, beginning at 1:100, in PBS were incubated with tissue for 30 minutes and then stained with the fluorescein-conjugated IgG fraction of monospecific goat antiserum to rabbit IgG (Cappel Laboratories, Inc., Malvern, Pa). To determine reactivity with rat MAC, we then incubated rabbit anti-rat MAC, diluted 1:100 in PBS, with equal volumes of PBS, fresh normal rat plasma containing 40 mM EDTA (lacking MAC), or zymosan-activated rat serum (containing MAC) for 1 hour at room temperature. After incubation the antibody was retitered by indirect immunofluorescence. Rabbit anti-rat MAC was subsequently used at the next to the last dilution which still gave positive staining of PHN tissue. Control sections were stained with normal rabbit IgG at the same concentration.

In addition to staining with rabbit anti-rat MAC and goat anti-rabbit IgG, described above, kidney tissue was stained with the fluorescein conjugated IgG fractions of monospecific rabbit anti-sheep IgG and anti-rat C3 and goat anti-rat IgG (Cappel Laboratories, Inc.). Normal rat kidney sections were also stained with these reagents. Stained sections were examined on a Leitz Ortholux II with a Ploempak 2.2 vertical fluorescence illuminator and appropriate filters for FITC fluorescence (E. Leitz, Inc., Rockleigh, NJ). Sections were pho-

Table 1—Results of Immunofluorescence and Protein Excretion

	Sheep IgG	Rat IgG	Rat C3	MAC	24-Hour urine protein excretion (mg)
PHN					
Heterologous phase (5 days; n = 5)	2–3+	tr	1–2+	2–3+	138 ± 27
CVF-treated (5 days; n = 3)	2–3+	tr	–	–	6.2 ± 0.6
γ_2-PHN					
Autologous phase (8 days; n = 4)	1–2+	1–2+	1–2+	2–3+	15.1 ± 7.2
CVF-treated (8 days; n = 3)	1–2+	1–2+	–	–	1.2 ± 0.2
AICN (7 weeks; n = 3)	NT	3–4+	1–2+	2–3+	36.7 ± 11.3
Nephrotoxic nephritis (1 day; n = 3)	3–4+	–	tr–1+	–	142 ± 48
Aminonucleoside nephrosis (9 days; n = 3)	–	–	–	–	268 ± 12
Segmental Sclerosis (24 weeks; n = 6)	2–3+	1–2+	2–3+	2–3+	260 ± 70

Mean ± SEM; tr, trace; –, negative; NT, not tested.

tographed on Kodak Ektachrome ED-135 film (Eastman Kodak Co., Rochester, NY) using an automatic exposure meter set at ASA-400. The intensity of fluorescent staining was reported semiquantitatively on a 0–4+ scale: 0, indistinguishable from control; trace, visible but cannot be photographed; 1+, definite but faint; 2+, definite; 3+, moderate intensity; and 4+, maximum intensity.

Other Procedures

Normal rat serum was activated with zymosan (Sigma Chemical Co., St. Louis, Mo) by incubating 1 part of a suspension containing 20 mg/ml of zymosan with 4 parts serum in a shaking water bath at 37 C for 30 minutes.

Urine protein excretion was measured on 24-hour urine collections obtained in metabolic cages. Protein content was measured by the sulfosalicylic acid method[27] with the use of a commercial standard (Labtrol, Dade Diagnostics, Inc., Aguado, Puerto Rico). Normal 24-hour urine protein excretion in age-matched rats in our laboratory is 2–6 mg.

Statistical Analysis of Data

All results of urine protein excretion determinations are expressed as mean ± SEM. The Mann-Whitney test was used for comparison of the results of protein excretion in different groups.[28] P values <0.05 are regarded as significant.

Results

Characterization of Anti-Rat MAC Antibody

Rabbit anti-rat MAC globulin made a single line of identity against a preparation of rat MAC (1 mg/ml) and zymosan-activated rat serum in micro-Ouchterlony immunodiffusion. In immunoelectrophoresis a single arc was seen against rat MAC. No reactivity was seen with EDTA–normal rat plasma in immunodiffusion or immunoelectrophoresis. In indirect immunofluorescence on PHN tissue, rabbit anti-rat MAC (30 mg/ml) had a titer of 1:1600. Incubation with PBS or EDTA-normal rat plasma did not alter the titer, whereas a single incubation with zymosan-activated serum decreased the titer to 1:200. Rabbit anti-rat MAC had no reactivity by indirect immunofluorescence with normal rat kidney.

Presence of MAC Deposits in Glomerular Disease Models

Passive Heymann Nephritis (Heterologous Phase)

Five days after injection of sheep anti-rat Fx1A antiserum all rats were proteinuric (138 ± 27 mg/day; Table 1). Immunofluorescence revealed 2–3+ finely granular deposits of sheep IgG and 1–2+ deposits of rat C3 in a subepithelial distribution as previously described.[9,20] Deposits of IgG were also present on proximal tubular brush borders and in a granular pattern along the tubular basement membranes. Faint deposits of C3 were seen on some tubular brush borders. Deposition of rat MAC was readily evident (2–3+) in a granular pattern similar to the distribution of IgG (Figure 1). Depletion of circulating complement components with CVF reduced proteinuria (6.2 ± 0.6; $P < 0.05$ versus controls; Table 1). Proteinuria in this model has previously been shown to be due to a complement-dependent, cell-independent mechanism.[9] Despite similar deposits of sheep IgG, deposition of rat C3 and MAC was prevented by CVF treatment (Figure 2).

γ_2-PHN (Autologous Phase)

Rats were immunized with sheep IgG prior to injection of γ_2-anti-Fx1A IgG in order to accelerate the development of the autologous phase. Eight days after the injection of antibody all animals were proteinuric

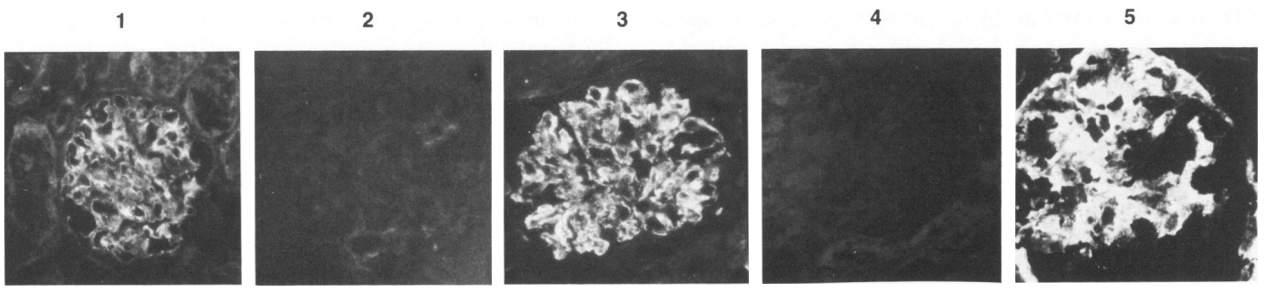

Figure 1—Granular deposits of rat MAC are seen along the glomerular capillary wall in the kidney of a proteinuric animal with PHN 5 days after the injection of sheep anti-Fx1A antiserum. (×400) **Figure 2**—Deposition of rat MAC is prevented in an animal with PHN after C depletion with CVF. (×400) **Figure 3**—Granular deposits of rat MAC, similar to those seen in PHN, are present in the glomerular capillary wall of an animal with AICN 7 weeks after immunization with Fx1A. (×400) **Figure 4**—Deposits of rat MAC are absent in an animal with severe proteinuria 24 hours after administration of sheep anti-rat GBM antiserum. (×400) **Figure 5**—Deposits of rat MAC are seen in the mesangium, on Bowman's capsule, and in sclerotic areas of an animal with developing segmental sclerosis 24 weeks after uninephrectomy and injection of sheep anti-rat Fx1A antiserum. (×400)

(15.1 ± 7.2 mg/day; Table 1). Immunofluorescence staining of kidney tissue revealed 1–2+ granular capillary wall deposits of sheep and rat IgG. Deposits of rat C3 (1–2+) and MAC (2–3+) were present in a similar distribution. Treatment of 3 rats with CVF again reduced proteinuria (1.2 ± 0.2 mg/day; Table 1). Proteinuria in this model has also been shown to be due to the same complement-dependent, cell-independent mechanism as in PHN.[8] Deposition of sheep and rat IgG was not altered by CVF treatment, but deposition of rat C3 and MAC was again prevented.

AICN

Seven weeks after immunization with Fx1A all rats were proteinuric (36.7 ± 11.3 mg/day; Table 1). Immunofluorescence showed diffuse 3–4+ subepithelial deposits of rat IgG and 1–2+ deposits of rat C3, as previously described.[22] Rat IgG deposits (1–2+) were also evident on proximal tubular brush borders, but no deposits were seen along the tubular basement membrane. All rats had positive staining in their glomeruli for MACs in a distribution similar to that of rat IgG and C3 (Figure 3). Because animals cannot be depleted of complement for the length of time necessary to induce this lesion, the role of complement in mediating proteinuria in AICN could not be defined.

Nephrotoxic Nephritis (NTN-Heterologous Phase)

All animals became proteinuric within the first 24 hours after injection of sheep anti-rat GBM antiserum (142 ± 48 mg/day; Table 1). Intense linear staining for sheep IgG (3–4+) was present diffusely on the glomerular basement membrane, but only trace–1+ deposits of rat C3 were present. No deposits of rat MAC could be detected in the kidneys of these animals (Figure 4). Proteinuria induced by the antiserum used in these experiments has previously been shown by us to be unaffected by CVF treatment.[16]

Aminonucleoside Nephrosis

Nine days after injection of aminonucleoside, rats had 268±12 mg/day of proteinuria. Immunofluorescence studies revealed focal 1+ mesangial deposition of rat IgG, similar to normal rat kidney, but no deposits of C3 or MAC. Treatment of such animals with CVF does not alter the development of proteinuria.[9]

Segmental Sclerosis

All animals were proteinuric after 24 weeks (260 ± 70 mg/day; Table I). Immunofluorescence showed 2–3+ finely granular capillary wall deposits of sheep IgG and 1–2+ deposits of rat IgG along the glomerular capillary wall. Segmental areas of sclerosis were present in the majority of glomeruli. Faint deposits of rat C3 were present in normal-appearing segments of the capillary wall, but large positively staining granules (2–3+) of C3 were present in sclerotic areas and in widened mesangial areas. Deposition of rat MAC (2–3+) was seen in the sclerotic areas, in widened mesangial areas, and on thickened portions of Bowman's capsule, with only 1+ deposits of MAC still present on the GBM (Figure 5).

Discussion

We examined several models of experimental glomerular injury in the rat for the presence of deposits of neoantigens of the MAC in an effort to provide further support for our hypothesis that the terminal complement components play an important role in the mediation of glomerular capillary wall injury. Antiserum to neoantigens of the rat MAC was produced in rabbits and its specificity assured by immunodiffusion and absorption studies. Renal tissue from animals with both immunologic and nonimmunologic renal injury were examined with the use of this antiserum; and where possible, the presence of the MAC was correlated with the

role of C in contributing to the injury. Three models of injury due to subepithelial deposits were examined. In the heterologous phase of PHN, deposits form when heterologous antibody binds directly to an antigen derived from glomerular epithelial cells,[29,30] and the resulting proteinuria requires C but not inflammatory cells for its production.[9] Animals with PHN had granular capillary wall deposits of MAC in a distribution similar to that of sheep IgG and rat C3 and corresponding to the location of the terminal C component deposits (C5, C6, C7, and C8), which we previously demonstrated in this model.[16] C depletion with CVF abolished proteinuria without altering sheep IgG deposition but prevented deposition of C3 and MAC.

In a second model of subepithelial-deposit–induced injury, in which γ_2 sheep IgG served as a planted antigen and deposits formed by the binding of rat anti-sheep IgG antibody to the sheep IgG, MAC deposition was again seen in the same distribution as sheep and rat IgG and rat C3. Proteinuria in this model is also C-dependent but cell-independent,[8] and C depletion again prevented proteinuria, C3, and MAC deposition without altering deposits of rat and sheep IgG. Thus, in these two models of C-mediated injury, proteinuric animals had glomerular deposits of MAC and C depletion prevented both proteinuria and MAC deposition. In animals with AICN, in which proteinuria is not seen for 6–10 weeks after immunization with Fx1A, deposits of MAC were present in a distribution similar to that of rat IgG. Because of the time necessary to produce this model, it is not possible to carry out C depletion during the entire period of production of capillary wall injury, and the role of C is unclear. However, we have previously demonstrated that deposits in this model occur in a manner and at a site similar to that of PHN, and C is likely to play a similar role in its pathogenesis.[31]

These results are consistent with our original hypothesis that the terminal C components play an important role in the genesis of injury in experimental membranous nephropathy and that the assembly of the membrane attack complex with subsequent damage to some element of the filtration barrier is involved. These observations are further strengthened by our findings in BSA-induced chronic serum sickness in rabbits, which in its early stages is predominantly a membranous lesion.[10,32] Proteinuria in this model was significantly reduced in C6-deficient rabbits, demonstrating a requirement for C6 in its pathogenesis.[10] Furthermore, deposits of human MAC have been found in renal biopsies of patients with both idiopathic and lupus membranous nephropathy.[12,15]

The above results do not exclude the possibility that deposition of MACs occurs secondary to tissue injury. Using cultured human kidney cells we have shown that cells damaged by a variety of insults when incubated in autologous serum activate C by the classic and alternative pathways, form cell-bound C3 convertases, and bind C5, C6, C7, and C8 to their surfaces.[33,34] In order to exclude this possibility, we examined two other models of glomerular injury in which C activation does not contribute to the development of proteinuria. The heterologous phase of sheep anti-rat GBM nephritis in our laboratory is characterized by heavy proteinuria without significant C3 deposition or neutrophil infiltration and no change in proteinuria following C depletion with CVF.[2] Although earlier studies of nephrotoxic nephritis in rabbits had suggested a role for C and neutrophils in its pathogenesis,[1,5,35] our results in rats are in agreement with those of Pilia et al.[36] In this model of C-independent antibody-induced glomerular disease severe degrees of proteinuria are not accompanied by deposits of neoantigens of the MAC. We have previously shown in this model that the individual terminal C components, C5, C6, C7, and C8, are also absent.[16] In contrast to these findings are results of a study of sheep anti-rabbit GBM nephritis in normal and C6-deficient rabbits which demonstrated complement deposits and a role for C6 and thus the involvement of the MAC in the production of proteinuria in that species.[11] In a nonimmunologic model of proteinuria produced by injection of aminonucleoside of puromycin, deposition of MACs does not occur. C depletion in this model has been shown to have no effect on proteinuria.[9] These findings suggest that tissue injury alone is probably not responsible for the finding of MACs in glomerular lesions.

Deposition of poly-C9, a component of the MAC, has also been reported in certain human renal diseases not believed to be of immunologic origin in which sclerotic changes are present in glomeruli, including hypertensive and diabetic nephrosclerosis.[13] The only experimental model of sclerosis which we examined, that which occurs in uninephrectomized animals with PHN, also displayed prominent deposits of MACs in widened mesangial areas and in areas of sclerosis as well as less intense deposits on the capillary wall. The importance of these deposits in the progression of sclerosis is unclear. Their appearance in mesangial areas early in the course of the disease (unpublished observations), together with the ability of damaged kidney cells to activate C *in vitro*, as described above, raises the possibility that they may contribute to the development of sclerotic lesions in progressive glomerular disease. Further studies in C-deficient strains of animals may help to resolve this question.

Taken together, these studies are consistent with the hypothesis that the terminal C pathway and the MAC play an important role in the pathogenesis of glomeru-

lar injury in several different forms of renal disease. These findings are also important in view of reports of neoantigens of the MAC and poly-C9 in several forms of human renal disease.[12,13,15] The mechanism by which MAC formation in the glomerular capillary wall might lead to an increase in permeability remains unclear. MACs are able to insert into a variety of cell membranes and lead to the formation of transmembrane channels, which contribute to cell lysis.[37,38] They have also been shown to produce release of lipids, including arachidonic acid, from membranes with a resultant stimulation of production of inflammatory mediators.[39] Although apparent insertion of MACs into cell-free alveolar basement membranes has been demonstrated,[40] it appears more likely that an interaction with glomerular epithelial cell membranes or slit pores might contribute to the altered permeability.[2,9] The nature of this mechanism requires further study.

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